10

15

20

25

-23-CLAIMS

- 1. A process for the preparation of an aqueous liquid comprising a phytase, the process comprising:
- (a) culturing in an aqueous medium a microorganism of the genus Aspergillus or Trichoderma having a heterologous phytase gene under the control of a glucoamylase (for Aspergillus) or cellobiohydrolase (for Trichoderma) promoter, under conditions that allow recombinant expression of the phytase, where the medium comprises, as a feed for the microorganism an assimilable carbon source and an assimilable nitrogen source;
- (b) filtering the aqueous medium to remove the microorganisms to give an aqueous filtrate; and
- (c) subjecting the filtrate from (b) to ultrafiltration to give an aqueous liquid, having a phytase concentration of at least about 14,000 FTU/g.
- 2. A process according to claim 1 where the microorganism is Aspergillus niger, Aspergillus oryzae or Trichoderma reesei.
- 3. A process according to claim 1 wherein the microorganism does not possess, or does not express, a glucdamylase (AG) gene.
- 4. A process according to claim 1 wherein the microorganism possesses multiple copies of the phytase gene.
- 5. A process according to claim 1 wherein the aqueous liquid is substantially free of taka-amylase.
- 6. A process according to claim I wherein substantially all of the carbon and nitrogen sources in the medium have been consumed by the microorganisms before filtering in (b).
- 7. A process according to claim 1 wherein the aqueous liquid is free of the carbon and/or nitrogen sources:
- 8. A process according to claim 1 wherein the phytase is expressed in the microorganism with a glucoamylase signal sequence.
- 9. A process according to claim 1 wherein neither the aqueous filtrate or the aqueous liquid are subjected to: (a) crystallisation; (b) a colour-removal step; or (c) crystallization and a colour removal step.

1

- 10. A process according to claim 1 wherein the resulting aqueous liquid has a phytase activity of about 18,000 FTU/g or more.
- An aqueous liquid prepared by a process according to claim 1 11. comprising a phytase at a concentration of at least about 14,000 FTU/g.
- 12. An aqueous liquid according to claim 11 which is derived from a culture medium in which the phytase was expressed.
- A process for the preparation of a phytase-containing granulate, 13. suitable for use in an animal feed, the process comprising processing a solid carrier comprising at least about 15% (w/w) of an edible carbohydrate polymer and an aqueous liquid according to claim 11 to obtain phytase-containing granules.
- 14. A process according to claim 13 that comprises mixing the aqueous liquid and carrier, and kneading the resulting mixture.
- A process according to claim 14 wherein the granules are subsequently 15. dried.
 - 16. A process according to claim 13 wherein the process comprises:
 - mixing the aqueous liquid/containing the phytase with the solid carrier; (a)
 - mechanically processing the mixture obtained in (a) to obtain enzyme-(b) containing granules; and
 - drying the enzyme-containing granules obtained in (b). (c)
- A process according to claim 15 wherein the processing comprises a 17. method selected from the group consisting of extrusion, pelleting, high-shear granulation, expansion, and fluid bed agglomeration.
- A phytase-containing granulate prepared by a process according to claim 13.
- **1**9. A granulate comprising dried granules formed from a phytase and a solid carrier which comprises at least about 15% (w/w) of an edible carbohydrate polymer.
- 20. A granulate according to claim 19 wherein the granules comprise at least one divalent cation.
- A granulate according to claim 19 wherein the granules comprise one 30 21. or more hydrophobic, gel-forming or water insoluble compound(s).

10

15

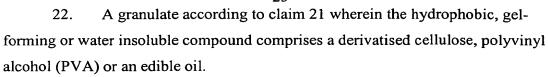
20

15

20

25

30



A granulate according to claim 22 wherein: (a) the derivatised cellulose is selected from the group consisting of hydroxy-propyl-methyl-cellulose, carboxy-methyl-cellulose and hydroxy-ethyl-cellulose; (b) the edible oil is soy oil or canola oil; or (c) the derivatised cellulose is as according to (a) and the edible oil is as according to (b).

- 24. A granulate according to claim 19 which additionally comprises an endo-xylanase and/or β-glucanase.
 - A granulate according to claim 19 wherein the carrier comprises 25. starch.
- 26. A granulate according to claim 19 wherein the phytase is other than a heat tolerant (thermostable) phytase.
- A granulate according to claim 19 wherein the phytase is a fungal 27. phytase.
 - A granulate according to claim 19 wherein the fungal phytase is 28. derived from an Aspergillus or Trichoderma species.
- A process for the preparation of an animal feed, a premix or precursor 29. to the animal feed, the process comprising mixing a phytase-containing granulate according to claim 19 with one or more animal feed substance(s) or ingredient(s).
- A process according to claim 29 wherein the mixture of feed substance(s) and composition or granulate is treated with steam, is pelletised, and optionally is dried.
 - A composition comprising: 31.
 - (a) a granulate according to claim 19;
- (b) a phytase-containing granulate with an activity of at least about 6,000 EXU/g; or
- (c) both a granulate according to (a) and a phytase-containing granulate according to (b).
- 32. A composition according to claim 31 which is an edible feed composition.
 - 33. A composition according to claim 31 which is an animal feed.

20

Jul 33

- 34. A composition according to claim 31, wherein said composition comprises pellets that comprise one or more feed substance(s) or ingredient(s) mixed with a granulate that comprises dried granules formed from a phytase and a solid carrier which comprises at least about 15% (w/w) of an edible carbohydrate polymer.
- 35. A composition according to claim 31 which is an animal feed, or a premix or precursor to an animal feed, and is prepared by a process that comprises mixing a phytase-containing granulate with one or more animal feed substance(s) or ingredient(s).
- 36. A process for promoting the growth of an animal, wherein said process comprises feeding an animal with a diet that comprises:
 - (a) a granulate according to claim 19;
 - (b) a phytase-containing granulate with an activity of at least about 6,000 FTU/g;
 - or (c) both a granulate according to (a) and a phytase-containing granulate according to (b).
 - 37. A process for promoting the growth of an animal, wherein said process comprises feeding said animal with a granulate as defined in claim 19 in, or as a component of, an animal feed, or in an animal diet.
 - 38. A method for improving the pelleting stability of phytase, wherein said method comprises formulating said phytase in a composition comprising at least about 15% (w/w) of an edible carbohydrate polymer as a carrier for said phytase.

